REMARKS

An Office Action was mailed in the above-captioned application on November 29, 2006. Claims 35-37 and 47-57 were pending in the application. Claims 35-37 and 47-57 were finally rejected. This Amendment and Remarks document is submitted in response to said Office Action.

Claim Objection

Claim 57 has been objected to as being dependent upon a rejected base claim. Applicant believes that Claim 57 would be allowed if amended to include all of the limitations of the base claim and any intervening claims. Applicant has canceled dependent Claim 57, and added new independent Claim 58, which includes all of the limitations of Claim 57 and the base Claim 35. It is respectfully submitted that Claim 58 is in condition for allowance.

The Rejection under 35 U.S.C. § 103(a)

The Examiner has rejected Claims 35-37 and 47-56 under 35 U.S.C. § 103(a) as being unpatentable. The Examiner bears the burden of establishing a *prima facie* case of obviousness (Section 103). In determining obviousness, one must focus on Applicant's invention as a whole. *Symbol Technologies Inc. v. Opticon Inc.*, 19 U.S.P.Q.2d 1241, 1246 (Fed. Cir. 1991). The primary inquiry is:

> whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have had a reasonable likelihood of success . . . . Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure.

In re Dow Chemical, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

A. The rejection over Gudin, et al., U.S. Pat. No. 5,179,012 in view of Wagner, et al., U.S. Patent No. 4,720,456. The Examiner has rejected Claims 35-37 and 47, 50, and 56 under 35 U.S.C. § 103(a) as being unpatentable over Gudin, et al., U.S. Pat. No. 5,179,012 in view of Wagner, et al., U.S. Patent No. 4,720,456. Specifically, the rejection contends that Gudin, et al. teaches methods for obtaining liposoluble components from microorganisms, comprising culturing the microorganism in a medium, grinding the microorganism and separating the phases

via centrifugation to obtain the desired components. The rejection further alleges that Gudin, et al., does not teach contacting the fermentation medium with a base, or wherein the temperatures are raised to at least 50°C, but that Wagner, et al., teaches methods for obtaining liposoluble components from microorganisms wherein the culture medium is adjusted with alkaline metal hydroxides and cultured at temperatures of 50°C, and that the addition of hydroxide increases the yield of lipids. The rejection reasons that it would have been obvious to culture microorganisms at about 50°C in the presence of hydroxides for the disclosed advantages of increased lipid yields.

Applicant respectfully traverses this rejection. There is no motivation to combine Gudin, et al., with Wagner, et al. Gudin, et al., teaches a method for culturing microalgae and cyanobacteria in a closed photobioreactor, and the recovery of antioxidants, such as the protein SOD therefrom. Wagner, et al., is concerned with optimizing the production of glycolipids, specifically trehalose lipids, in a fermentation of bacteria that produce such lipids. Gudin, et al., teaches maximizing growth of the microorganisms by using high oxygen conditions. Wagner, et al., in contrast, teaches that a limitation of the growth rate is advantageous for increased production of glycolipids. Limitation of the growth rate is accomplished by, for example, addition of hydrocarbon to the growth medium, addition of a complex-former to the growth medium, limitation of nitrogen in the growth medium, and addition of a bacteriostatic agent to the growth medium. One skilled in the art would not be motivated to apply the methods of Wagner, et al., directed to increasing the production of glycolipids in glycolipid-producing bacteria, with the methods of Gudin, et al., which are directed toward recovering liposoluble antioxidants (but not lipids themselves) from a closed photobioreactor system. Since there is no motivation to combine, Gudin, et al., with Wagner, et al., Applicants submit that a *prima facie* case of obviousness has not been made. The rejection should be withdrawn on this basis alone.

Even if it were proper to combine Gudin, et al., with Wagner, et al., which Applicants do not admit, it is respectfully submitted that Gudin, et al. does not disclose all the elements of independent Claim 35, and that Wagner, et al., does not remedy the deficiencies of Gudin, et al., Applicant therefore submits, as explained in detail below, that the combination of Gudin, et al., and Wagner, et al. can not render obvious Claims 35-37.

Claim 35 requires, in step c), increasing the temperature of the fermentation broth to at least about 50°C to lyse cells of said microorganisms to produce a lysed cell mixture, and in step

d) separating substances of different densities from said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer and wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids. Gudin, et al., does not teach such steps. Rather, Gudin, et al., teaches separation of microorganisms from the fermentation medium, crushing the microorganisms, and adding a solvent to the resulting cellular suspension. (col. 2., lines 47-53; col. 3, line 49 to col. 4, line 13). This mixture can then be separated into phases. There will be two or three phases depending on whether an aqueous solvent was used (col. 2., lines 55-56; col. 3., lines 14-19). The solvent functions to solubilize antioxidants produced by the microorganisms. In the typical solvent extraction process described by Gudin, et al. (see column 2, lines 51-53; column 4, lines 3-21 and column 5, line 56-column 6, line 11), the desired product, antioxidants, preferentially migrate to the solvent phase and can subsequently be recovered. The phases that Gudin, et al., references are a liquid phase (or phases) and a solid phase (col. 2, line 54).

The present invention is performed with a lysed cell mixture resulting from lysing cells in the fermentation broth. Gudin, et al., neither teaches nor suggests such a step, and in fact, teaches that the broth and the cells should be treated separately (col. 3, lines 54-58). Gudin, therefore, cannot obviate Claim 35 or any claim dependent therefrom.

Additionally, the presence of emulsified lipids in a light layer in Gudin, et al., is not taught. The rejection asserts (page 8) that Gudin, et al. teaches a process that produces phases which are separated into multiple layers and that lipid can be purified from the layers. Applicants respectfully point out, however, that Gudin, et al. does not teach purification of lipids, but rather, the recovery of liposoluble antioxidants. The liposoluble antioxidants of Gudin, et al. are not lipids themselves.

The rejection also asserts (page 8) that if the steps of Gudin, et al. and the present invention are the same, the lipids must be present in the phase separated layer. As explained above, however, the steps are not the same. Specifically, Gudin, et al., adds solvent to a cell suspension that has been separated from its fermentation medium to generate phases. The number of phases generated is a function of the type of solvent used, not the properties of the fermentation broth/lysed cell mixture. Gudin, et al., however, always generates a solid phase which contains the cellular debris. Solvent addition also solubilizes liposoluble antioxidants. In the present invention, there is no reason to solubilize liposoluble antioxidants or remove cellular

debris in a solid phase. Rather, in the present invention, lipid is efficiently and effectively separated from the fermentation broth/lysed cell mixture based on density, e.g., by centrifugation. As a result, the cellular debris resides in the aqueous phase, rather than a solid phase, and the desired lipid product is in its own lipid-rich phase, rather than dissolved or dispersed in an added solvent phase. The steps of Gudin, et al., result in different phases/layers than the steps of the present invention. Furthermore, even if lipids are present in some phase or layer which is taught by Gudin, et al., there is no teaching or suggestion that the lipids are emulsified. Applicants submit that when a solvent is used in the method of Gudin, et al., that the lipids will be soluble in the solvent; that is, they will dissolve in the solvent rather than be emulsified. Gudin, et al., cannot be seen as teaching or even suggesting a layer comprising emulsified lipids.

Furthermore, the present invention teaches dissolving proteins in step b) of Claim 35 because the inventors identified them as a component responsible for maintaining the emulsion of lipids and water that hinders recovery of the lipids. Gudin, et al., on the other hand, tries to recover the proteins. (The rejection at page 8 suggests that Applicant asserted that Wagner is recovering proteins; however, Applicant stated that Gudin, et al., recovers proteins). Gudin, et al., teaches recovery of SOD, an enzyme (protein) and therefore teaches conditions that protect proteins rather than those that denature or hydrolyze proteins.

In view of the differences between the present invention and Gudin, et al., Applicant submits that Gudin, et al., neither teaches nor suggests the present invention.

Wagner, et al. does not remedy the deficiencies of Gudin, et al., since Wagner, et al., also does not teach or suggest separating substances of different densities from said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids. Thus, the teachings of Gudin, et al., and Wagner, et al., when taken together, do not teach or suggest separating substances of different densities from said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids, as required by Claim 35.

Furthermore, Claim 35 requires in step b), contacting said fermentation broth with a base to dissolve at least a part of any proteins present in said fermentation broth. Wagner, et al.

teaches the addition of alkaline metal hydroxides to maintain the pH of a growing culture at a selected value (pH=3-8) in order to limit the source of nitrogen in the culture (col. 4, lines 29-30, and col. 5, lines 48-57). The rejection (page 6) states that it would be obvious to one skilled in the art to culture microorganisms at about 50°C in the presence of hydroxides for the disclosed advantages of increased lipid yields. Later, at page 8, the rejection states that Wagner, et al., adds a base to "increase the recovery of lipids." It must be emphasized that the conditions referred to in Wagner, et al., are conditions for optimizing the production of glycolipids in a growing culture. In general, increasing the production of glycolipids is accomplished by stressing the microorganisms to limit their total growth. In contrast, a lipid recovery process or isolation process occurs after the fermentation. Wagner, et al., teaches that recovery of glycolipids is accomplished by a known solvent extraction procedure (col. 6, lines 9-16, and Example 1). Thus, while Wagner, et al., may teach that pH control of a growing culture may be advantageous for increasing glycolipid production in the growing cells, Wagner, et al., do not teach anything about the pH during the recovery process. Indeed, if one were to dissolve proteins using a base as claimed in step b of Claim 35 and lyse cells at certain temperatures as claimed in step c of Claim 35, it would kill the growing cells of the Wagner, et al. culture which is certainly not what the Wagner, et al. reference teaches or suggests.

The present invention does not teach the addition of base to maintain the pH of a growing culture, but rather to dissolve protein, which interrupts the biochemical processes of the microorganisms. The addition of base in Wagner, et al., and the addition of base in the presently claimed invention are made for vastly different purposes. Wagner, et al., adjusts pH during a fermentation to promote glycolipid metabolism, while in the present invention the pH is raised with a base to dissolve proteins; such a pH will slow or stop growth of the microorganisms. The rejection asserts (page 8) that while Wagner, et al., does not specifically dissolve proteins, such an action is intrinsic to the method since the method steps are the same. Applicant, however, disagrees that the method steps are the same. Wagner, et al., teaches that the hydroxide is added to limit the nitrogen content of the culture suspension to the amount which is dissolved in the initially added nutrient salt solution. (col. 5, lines 48-57). The hydroxide is added "to maintain the pH value of the culture suspension at the selected value," which is taught to be in the acid to neutral range of pH=3-8. In the present invention, the base is added "to dissolve proteins,"

which would not be in an acid or neutral range and which would not be effective in limiting the nitrogen content of the medium.

Thus, the teachings of Gudin, et al., and Wagner, et al., when taken together, do not teach or suggest contacting said fermentation broth with a base to dissolve at least a part of any proteins present in said fermentation broth, as required by step b) of Claim 35.

With regard to the rejection's statement that Wagner, et al. teaches culturing microorganisms at temperatures of 50°C, this teaching also does not obviate the present invention. The rejection (page 6) states that it would be obvious to one skilled in the art to culture microorganisms at about 50°C for the disclosed advantages of increased lipid yields. As noted above, the "increased lipid yields" of Wagner are increases in the glycolipid production in a growing culture. Wagner, et al., does not teach or suggest any temperature control in the glycolipid isolation or recovery process.

Again, Wagner, et al., teaches growth or cultivation of microorganisms from 15°C to 50°C, or below 50°C (col. 4, lines 23-31). Thus, in Wagner, the temperature of 50°C represents an upper limit for the growth or fermentation conditions of the microorganisms. In contrast, the present invention does not teach or require culturing microorganisms at 50°C; rather, Claim 35 requires increasing the temperature of said fermentation broth to at least about 50°C to lyse cells of said microorganisms to produce a lysed cell mixture. In other words, in the present invention, 50°C represents a lower limit for the temperature used to lyse, i.e., kill, the cells in a method to obtain lipids from microorganisms. Neither Wagner, et al., nor Gudin, et al. teach increasing the temperature of a microorganism fermentation broth to at least about 50°C to lyse the cells. Thus, the teachings of Gudin, et al., and Wagner, et al., when taken together, do not teach or suggest step c) of Claim 35.

Finally, the rejection states (page 4) that although the references do not specifically teach multiple separation of phases, it would have been well within the purview of one in the art to optimize the number of washings and separations as a matter of routine experimentation. It is well settled that the Examiner can not rely on general conclusions of "basic knowledge" or "common sense." Rather, evidence is required. *In re Lee*, 277 F.3d 1338, 1345-46 (Fed. Cir. 2002). In the present case, no evidence has been presented to support this assertion. Furthermore, the present claims require adding an aqueous washing solution. Since both Gudin,

et al., and Wagner, et al., utilize solvent extraction methods, it is submitted that an aqueous washing solution is neither taught not suggested by either reference, or the combination thereof.

For the foregoing reasons, Applicant submits that the combination of Gudin, et al., and Wagner, et al., cannot obviate the present invention and respectfully requests reconsideration of the rejection under 35 U.S.C. § 103.

B. The rejection over Gudin, et al., U.S. Pat. No. 5,179,012 in view of Wagner, et al., U.S. Patent No. 4,720,456 and Barclay, U.S. Patent 5,130,242. The Examiner has rejected Claims 35-37 and 47, 50, and 56 under 35 U.S.C. § 103(a) as being unpatentable over Gudin, et al., U.S. Pat. No. 5,179,012 in view of Wagner, et al., U.S. Patent No. 4,720,456 and Barclay, U.S. Patent 5,130,242.

Barclay teaches a process for the production of microbial products with high concentrations of omega-3 highly unsaturated fatty acids, preferably using microorganism of the order Thraustochytriales. The teachings of Gudin, et al., and Wagner, et al. have been discussed above. Barclay does not remedy the deficiencies of Gudin, et al., and Wagner, since Barclay also does not teach or suggest separating substances of different densities from said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, and wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids. The combination of references, therefore, cannot obviate the present claims.

Reconsideration of the rejection under 35 U.S.C. § 103 is respectfully requested.

Closing Remarks

Applicant believes that the pending claims are in condition for allowance. The Examiner is invited to call the undersigned to schedule a telephone interview to resolve any remaining issues in this application.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-1970, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-1970.

Respectfully submitted,

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